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WO 00/35443 A1

(54) Title: METHOD AND COMPOSITION FOR THE MAINTENANCE AND RESTITUTION OF GUT INTEGRITY

(57) Abstract: A method, and formulation for use in the method, for maintaining and enhancing the restitution of gut integrity in an individual in need thereof comprising administering to the individual a formulation comprising an effective amount at least one n-6 polyunsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid.

METHOD AND COMPOSITION FOR THE MAINTENANCE AND RESTITUTION OF GUT INTEGRITY

FIELD OF THE INVENTION

The present invention is directed to enteral formulations that contain long-chain polyunsaturated fatty acids (PUFAs) and to methods for maintaining gut integrity and enhancing the restitution of gut integrity. More specifically the present invention is directed to formulations and methods for reducing gut permeability and restoring damaged intestinal cells to maintain and enhance the restitution of gut integrity.

BACKGROUND OF THE INVENTION

Several disease states are characterized by an increase in gut permeability. Examples of such disease states include colitis, Crohn's disease, irritable bowel syndrome, celiac disease, starvation, cystic fibrosis, necrotizing enterocolitis and gut damage resulting from chemotherapy and radiation treatment. Moreover, food allergies in the premature infant and/or an immature gut is associated with a higher permeability predisposing the infant to aberrant nutrient absorption and translocation of endotoxins and bacteria into the circulation.

Long chain PUFAs in enteral formulations or compositions are well known. U.S. Patent No. 4,670,285 discloses a specific fat blend suitable for use in infant formulations. The fat blend contains at least one C_{20} or C_{22} n-6 fatty acid and one C_{20} or C_{22} n-3 fatty acid. The C_{20} or C_{22} n-6 fatty acid is present in a total amount of about 0.13 to 5.6% by weight of all fatty acids in the composition. The C_{20} or C_{22} n-3 fatty acid is included in a total amount of about 0.013 to about 4.44% by weight of all fatty acids in the composition. This patent discloses the use of egg lipids to supply the fatty acids.

U.S. Patent No. 4,918,063 discloses formulations containing mixtures of phospholipids and neutral lipids for the prevention or treatment of ulcers and inflammatory bowel disease. This patent discloses a mixture of saturated or unsaturated phospholipids, together with saturated or unsaturated triglycerides and/or sterols as providing ulcer protection in experimental animal models.

PCT International Publication No. WO96/10922 discloses a fat mixture for infant formula characterized in that arachadonic acid and docosahexanoic acid are present in the form of phospholipids.

European Patent Application No. 0376628 B1 discloses an all vegetable oil fat blend which utilizes randomized palm oil or randomized palm olein oil as the sole palmitic acid oil source. It is disclosed that the compositions are particularly useful in infant formulas particularly for preterm or low birth weight infants.

SUMMARY OF THE INVENTION

The present invention is directed to methods for maintaining gut integrity and enhancing the restitution of gut integrity in an individual in need thereof, comprising administering to the individual a formulation comprising at least one n-6 poly-unsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid, to formulations for use in the method comprising an effective amount of at least one n-6 polyunsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid, and the to use of an effective amount of at least one n-6 polyunsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid in the manufacture of a medicament for use in maintaining gut integrity and enhancing the restitution of gut integrity in an individual in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 compares plasma endotoxin levels in rats supplemented with the formulation of the invention to rats that have not been supplemented.

Figure 2 shows the effect of the present formulation on intestinal PLA_2 mRNA expression levels.

Figure 3 shows the effect of the present formula on intestinal PAF-receptor levels in RNA.

DETAILED DESCRIPTION OF THE INVENTION

The polyunsaturated fatty acids useful in the present invention include fatty acids of twenty carbons or more having at least two carbon-carbon double bonds. The number and position of double bonds in the fatty acids are designated by the

nomenclature conventionally used. For example, arachidonic acid has a chain length of 20 carbon atoms and 4 double bonds beginning at the sixth carbon from the end of the molecule. Accordingly, arachidonic acid is referred to as C_{20}:4 n-6. Similarly, docosahexanoic acid has a chain length of 22 carbon atoms with 6 double bonds beginning at the third carbon from the end of the molecule and is designated C_{22}:6 n-3.

Preferably, the n-6 polyunsaturated fatty acid used in the present invention is arachidonic acid and the n-3 polyunsaturated fatty acid used in the present invention is docosahexanoic acid.

The method of the present invention comprises administering to an individual having one or more disorders characterized by cell damage or destruction to gut tissue an effective amount of a formulation containing at least one n-6 polyunsaturated fatty acid and at least one n-3 polyunsaturated fatty acid. Effective amounts of the n-6 polyunsaturated fatty acid is about 5 to 61 mg and preferably 10 to 51 mg/kg body weight. Similarly, effective amounts of the n-3 polyunsaturated fatty acid is about 5 to 61 and preferably 5 to 51 mg/kg body weight. The effective arachidonic acid: docosahexanoic acid ratio is about 1:1 to 2.5:1, and preferably 1:1 to 2:1. This is based on administration of 100 kcal/kg/day for a 1 kg infant.

In order to achieve the effective amounts of n-6 and n-3 polyunsaturated fatty acid indicated above, the formulations used in the present method should comprise about 4 to about 50, and preferably about 8 to about 41 mg/100 ml of polyunsaturated fatty acid. The formulations used in the present method should comprise about 4 to about 50, and preferably about 4 to about 41 mg/100 ml of the n-3 polyunsaturated fatty acid. Again, the effective arachidonic acid: docosahexanoic acid ratio is 1:1 to 2.5:1 and preferably 1:1 to 2:1.

The present invention is directed to methods for maintaining gut integrity and enhancing the restitution of gut integrity, to formulations for use in for maintaining gut integrity and enhancing the restitution of gut integrity and to the use of an effective amount at least one n-6 polyunsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid in the manufacture of a medicament for use in for maintaining gut integrity and enhancing the restitution of gut integrity. Gut integrity is compromised in a number of disease states which increase gut

permeability, cause cell damage or destruction in the gut and cause bacterial translocation in the gut which may lead to endotoxemia. Disease states which compromise gut integrity in this manner include colitis, Crohn's disease, irritable bowel syndrome, celiac disease, starvation, cystic fibrosis, damage resulting from chemotherapy and radiation treatment and food allergies. In the premature infant, the immature gut is also associated with higher permeability which predisposes the infant to aberrant nutrition absorption and translocation of endotoxin and bacteria into the circulation. The present formulation is administered enterally to both adults and infants.

A preferred embodiment of this invention is a method or formulation for maintaining and enhancing the restitution of gut integrity in infants, particularly preterm infants. This method comprises enterally administering to the infants a nutritionally complete infant formula comprising proteins, carbohydrates, lipids and effective amounts of at least one n-6 polyunsaturated fatty acid and at least one n-3 polyunsaturated fatty acid.

The term 'infant formula' will be readily recognizable to those skilled in the art. When diluted or reconstituted, if initially in concentration or powder form, to the ready to feed state, a typical infant formula will comprise about 60-110 grams of carbohydrates per liter, 10-35 grams of protein per liter, and 20-50 grams of lipid per liter, as well as vitamins, minerals, fibers, emulsifiers, etc. To such an infant formula is added the appropriate amounts of n-6 and n-3 polyunsaturated fatty acid to maintain and enhance the restitution of gut integrity in the infant in need of treatment. Examples of suitable, commercially available infant formula to which the n-6 and n-3 polyunsaturated fatty acid may be added include S-26, S-26 LBW and SMA available from Wyeth Nutritionals International.

Specific examples of fat blends containing appropriate amounts of n-6 and n-3 polyunsaturated fatty acids according to the present invention are set forth below:

Fat Blend 1

	Fatty Acid	Fatty Acid	%
	C6:0	Caproic	--
	C8:0	Caprylic	9.1
	C10:0	Capric	6.1
	C12:0	Lauric	10.3
	C14:0	Myristic	4.6
	C14:1	Myristoleic	--
	C16:0	Palmitic	13.5
	C16:0 at sn-2	*% of Palmitic*	
	C16:1w7	Palmitoleic	0.1
	C18:0	Stearic	4.8
	C18:1w9	Oleic	32.3
	C18:2w6	Linoleic	15.9
	C18:3w3	Linolenic	1.7
	C20:0	Arachidic	0.3
	C20:2w6		--
	C20:3w6		--
	C20:4w6	Arachidonic	0.6
	C22:0	Docosanoic	0.3
	C22:6w3	Docosahenxaenoic	0.4
	C24:0		
	Total		100

Fat Blend 2

	Fatty Acid	Fatty Acid	%
	C6:0	Caproic	0.35
	C8:0	Caprylic	9.87
	C10:0	Capric	4.63
	C12:0	Lauric	6.4
	C14:0	Myristic	2.46
	C14:1	Myristoleic	0.02
	C16:0	Palmitic	13.43
	C16:0 at sn-2	*% of Palmitic*	*39.0*
	C16:1w7	Palmitoleic	0.03
	C18:0	Stearic	2.61
	C18:1w9	Oleic	39.39
	C18:2w6	Linoleic	13.94
	C18:3w3	Linolenic	1.29
	C20:0	Arachidic	0.01
	C20:2w6		0.01
	C20:3w6		0.02
	C20:4w6	Arachidonic	0.61
	C22:0	Docosanoic	0.3
	C22:6w3	Docosahenxaenoic	0.38
	C24:0		0.02
	Total		100

Specific examples of infant formula suitable for use in the present invention are set forth below:

Infant Formula A

Ingredients	%
Water	84.30
Lactose	1.20
Nonfat Dry Milk	2.83
Whey Powder	2.83
Maltodextrin	4.45
Fat Blend	4.11
Vitamins	0.02
Minerals	0.25
Taurine	0.01
	100.00

Infant Formula B

Ingredients	%
Water	57.77
Skim Milk (liquid)	29.49
Whey Protein Concentrate	2.76
Lactose	1.16
Maltodextrin	4.35
Fat Blend	4.15
Vitamins	0.03
Minerals	0.28
Taurine	0.01
	100.00

The present invention will now be illustrated with reference to the following specific example.

EXAMPLES

The method of the present invention was tested in a neonatal rat model of hypoxia induced gut injury. Reduced gut permeability is associated with reduced endotoxemia and a lowered intestinal phospholipase-2 (PLA_2) gene expression. Lower PLA_2 expression levels is indicative of a down-regulation of the inflammatory cascade.

Animal Model. Rat pups were delivered via abdominal incision from time-dated pregnant Sprague-Dawley rats on the 21st day of gestation. The newborn rats were fed via the orogastric route 0.1 ml of formula (S26-LBW formula, available from Wyeth Nutritionals International, Burlington, VT) every three hours with the volume advanced as tolerated up to 0.4 ml by 72 hours of life. In addition, animals were stressed with asphyxia twice daily by breathing 100% nitrogen gas for 5.0 seconds and then cold exposure to 4°C for 10 minutes. Using this protocol, 70-80% of animals develop abdominal necrosis by the 3rd to 4th day of life as described in Caplan, M.S.; Hedlund, E.; Adler, L.; and Hsueh, W., "Role of Asphyxia and Feeding in a Neonatal Rat Model of Necrotizing Enterocolitis," Pedatr. Pathol., 14:1017-1028 (1994).

Treatment Groups. There were 2 treatment groups: preterm formula (Formula A) and preterm formula with LCPUFAs (Formula B). The LCPUFAs added to Formula B were arachidonic acid and docosahexanoic acid. The LCPUFAs were added in the following amounts: 20 mg/100kcal docosahexanoic acid and 30 mg/100 kcal arachidonic acid. The first component of the study examined gut damage which was confirmed at time of necropsy by gross examination and histopathology. Gross examination and histopathology characterized changes in gut damage, maturity and integrity. The second component of the study investigated the mechanisms underlying changes in gut damage, maturity and integrity. The following outcome variables were measured: endotoxemia and PLA_2.

Gut Damage. Following euthanasia, the intestines were evaluated by gross inspection and histologic evaluation for confirmation of gut damage and maturation.

Endotoxemia. Plasma endotoxin was assessed using a spectrophotometric method provided in a Limulus Amebocyte Lysate (LAL) assay commercially available from Biowhittaker, Walkersville, MD.

Phospholipase A_2 mRNA expression. To quantitate PLA_2 mRNA expression, competitive PCR was performed using mRNA recovered from intestinal homogenate. Serial dilutions of cRNA were added to intestinal RNA and the PCR reaction was performed using standard conditions previously described. Products were run on an agarose gel and then quantified using phosphorimaging density.

Statistics. Differences between groups of ordinate data were compared using Chi-square analysis using Yates' correction. Differences between continuous variables were compared using the Student-t test or analysis or variance where appropriate. $P < 0.05$ was considered significant.

Supplementation with LCPUFAs reduced the incidence of ischemic gut damage (ischemic gut damage: 17/24 control vs. 8/24 LCPUFA, $p = 0.031$ using 3x2 chi-square) compared to the formula without LCPUFAs in neonatal rats (see Table 1). Animals developed symptoms of intestinal injury typically between 48 and 72 hours of life, and included abdominal distention, discoloration of the anterior abdominal wall, bloody stools, and respiratory distress.

Table 1

Effect of formula supplementation on reducing ischemic gut damage*

Formula I.D.	Ischemic Gut Damage	No Ischemic Gut Damage
A	17	7
B	8	16

*P = .031

As seen in Figure 1, plasma endotoxin levels measured between 48-72 hours showed that LCPUFA supplemented rats had lower plasma endotoxemia (25 ± 4 EU/ml) than the unsupplemented rats (276 ± 39 EU/ml). Figure 2 demonstrates that Intestinal PLA_2 mRNA expression was significantly lower among LCPUFA supplemented rats (0.41 + 0.09 molecules/gm tissue) as compared to unsupplemented rats (0.68 ± 0.11 molecules/gm tissue). Moreover, intestinal PAF receptor mRNA expression was also lower among LCPUFA supplemented rats, 1.5 ± 0.3 vs. 2.1 ± 0.2 (Figure 3).

The results demonstrate that LCPUFA supplementation reduces gut injury in an established neonatal rat model of ischemic gut damage. Furthermore, the data demonstrate that the beneficial effect of LCPUFA supplementation includes reduced gut permeability. The reduced gut permeability was associated with a reduction in endotoxemia. Endotoxemia is a result of bacterial and/or endotoxin translocation across the gut into the systemic circulation. Concurrent lower expressions of intestinal PLA_2 mRNA and PAF receptor mRNA were indicative of a reduced endotoxin induced inflammatory response.

The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indicating the scope of the invention.

WHAT IS CLAIMED IS:

1. A method for maintaining and enhancing the restitution of gut integrity in an individual in need thereof comprising administering to the individual a formulation comprising an effective amount at least one n-6 polyunsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid.

2. A method according to claim 1, where said n-6 polyunsaturated fatty acid is arachidonic acid and said n-3 polyunsaturated fatty acid is docosahexanoic acid.

3. A method according to claim 1 or 2, where said formulation is an infant formula.

4. A method according to any of claims 1 to 3, wherein said formulation comprises 4 to 50 mg/100 ml of said polyunsaturated n-6 fatty acid and 4 to 50 mg/100 ml of said polyunsaturated n-3 fatty acid.

5. A method according to claim 4, wherein said formulation comprises 8 to 41 mg/100 ml of said n-6 polyunsaturated fatty acid and 4 to 41 mg/100 ml of said n-3 polyunsaturated fatty acid.

6. A method according to any preceding claims, wherein said formulation comprises said n-6 polyunsaturated fatty acid and said n-3 polyunsaturated fatty acid in a ratio of 1:1 to 2.5:1.

7. A method according to claim 6, wherein said formulation comprises said n-6 polyunsaturated fatty acid and said n-3 polyunsaturated fatty acid in a ratio of 1:1 to 2:1.

8. A formulation comprising an effective amount at least one n-6 polyunsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid for use in maintaining and enhancing the restitution of gut integrity in an individual in need thereof.

9. A formulation according to claim 8, where said n-6 polyunsaturated fatty acid is arachidonic acid and said n-3 polyunsaturated fatty acid is docosahexanoic acid.

10. A formulation according to claim 8 or 9 where said formulation is an infant formula.

11. A formulation according to claim any of claims 8 to 10 wherein said formulation comprises 4 to 50 mg/100 ml of said polyunsaturated n-6 fatty acid and 4 to 50 mg/100 ml of said polyunsaturated n-3 fatty acid.

12. A formulation according to claim 11 wherein said formulation comprises 8 to 41 mg/100 ml of said n-6 polyunsaturated fatty acid and 4 to 41 mg/100 ml of said n-3 polyunsaturated fatty acid.

13. A formulation according to any of claims 8 to 12, wherein said formulation comprises said n-6 polyunsaturated fatty acid and said n-3 polyunsaturated fatty acid in a ratio of 1:1 to 2.5:1.

14. A formulation according to claim 13, wherein said formulation comprises said n-6 polyunsaturated fatty acid and said n-3 polyunsaturated fatty acid in a ratio of 1:1 to 2:1.

15. Use of an effective amount at least one n-6 polyunsaturated fatty acid in combination with at least one n-3 polyunsaturated fatty acid in the manufacture of a medicament for use in maintaining and enhancing the restitution of gut integrity in an individual in need thereof.

16. Use according to claim 15, where said n-6 polyunsaturated fatty acid is arachidonic acid and said n-3 polyunsaturated fatty acid is docosahexanoic acid.

17. Use according to claim 15 or 16 where said formulation is an infant formula.

18. Use according to any of claims 15 to 17 wherein said formulation comprises 4 to 50 mg/100 ml of said polyunsaturated n-6 fatty acid and 4 to 50 mg/100 ml of said polyunsaturated n-3 fatty acid.

19. Use according to claim 18, wherein said formulation comprises 8 to 41 mg/100 ml of said n-6 polyunsaturated fatty acid and 4 to 41 mg/100 ml of said n-3 polyunsaturated fatty acid.

20. Use according to claim 19, wherein said formulation comprises said n-6 polyunsaturated fatty acid and said n-3 polyunsaturated fatty acid in a ratio of 1:1 to 2.5:1.

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21. Use according to claim 20, wherein said formulation comprises said n-6 polyunsaturated fatty acid and said n-3 polyunsaturated fatty acid in a ratio of 1:1 to 2:1.

Effect of formula on plasma endotoxin

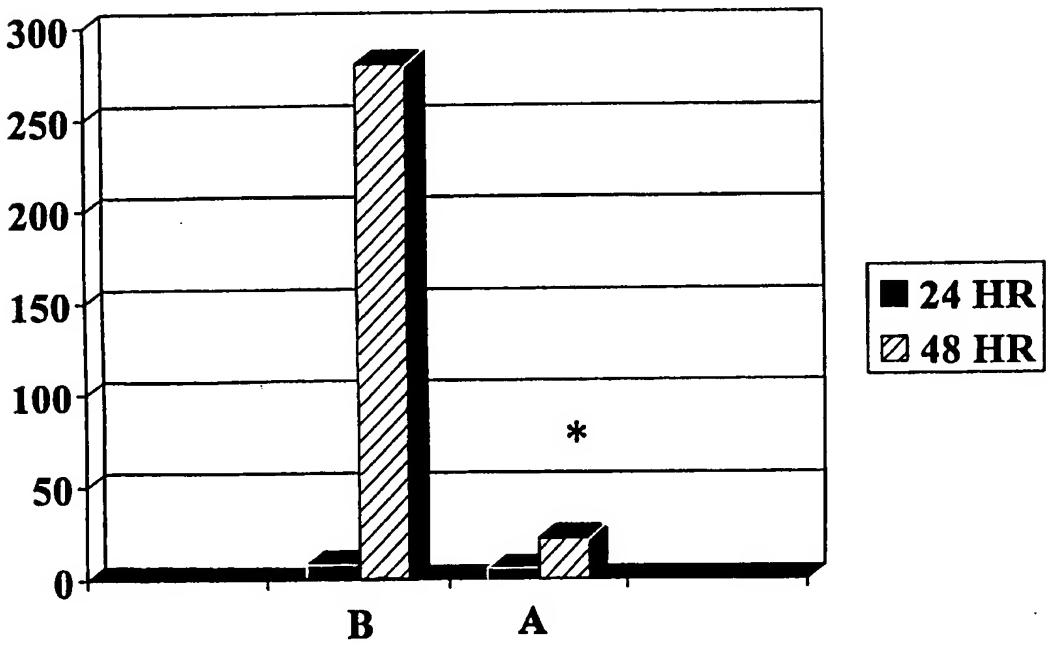
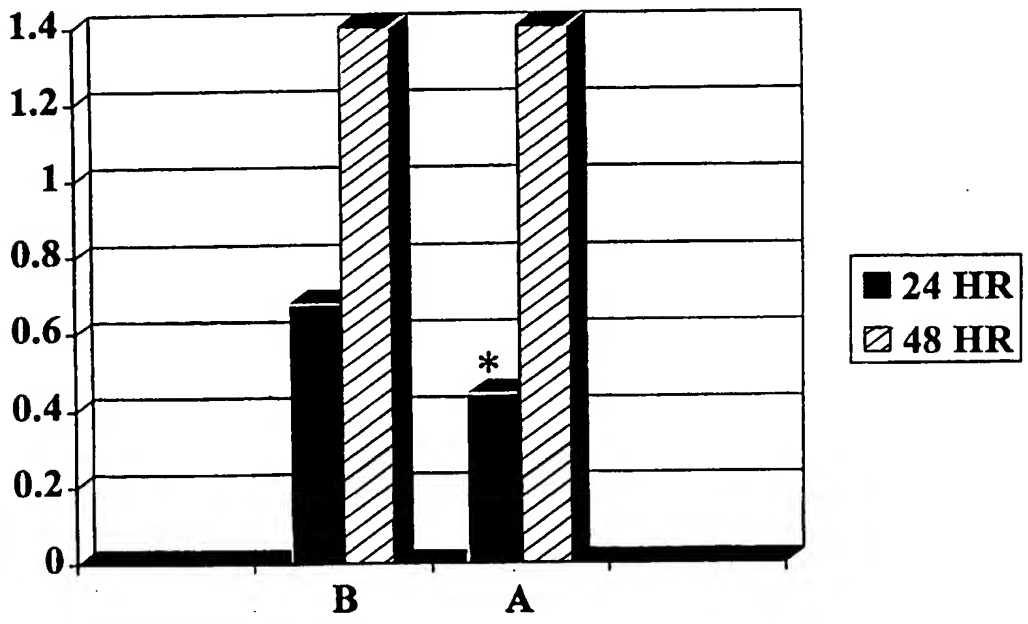


FIG. 1

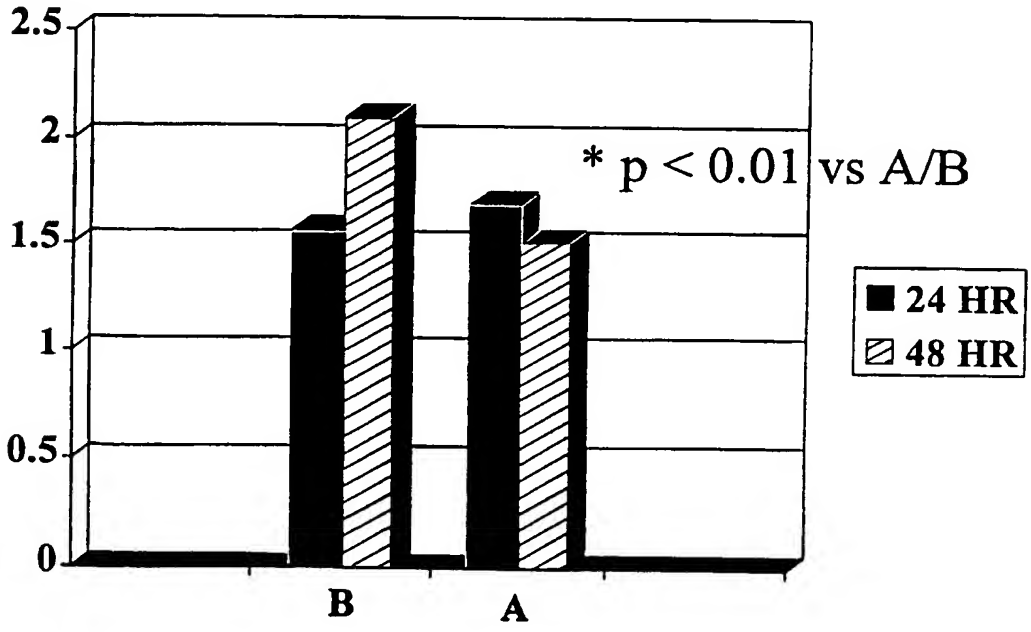
Effect of formula on intestinal PLA_2 mRNA expression



RT-PCR using B-actin as control

FIG. 2

Effect of formula on intestinal PAF-receptor mRNA



RT-PCR using GAPDH as control

FIG. 3

INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/US 99/29478

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/20 A61P1/00 A23L1/30 A23C11/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L A23C A23D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 733 360 A (SCOTIA HOLDINGS PLC) 25 September 1996 (1996-09-25) claims; examples 4-6	1,2,6-9, 13-16, 20,21
X	EP 0 711 503 A (SCOTIA HOLDINGS PLC) 15 May 1996 (1996-05-15) abstract page 2, line 3 -page 3, line 58; claims; examples	1-21
X	WO 98 48646 A (BETH ISRAEL HOSPITAL) 5 November 1998 (1998-11-05) the whole document	1-21
X	US 5 444 054 A (GARLEB KEITH A ET AL) 22 August 1995 (1995-08-22) the whole document	1-21

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 April 2000	04. 05. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Boddaert, P

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/29478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CARNIELLI V.: "Intestinal absorption of long chain polyunsaturated fatty acids in preterm infants fed breast milk or formula" AMERICAN JOURNAL OF CLINICAL NUTRITION, vol. 67, no. 1, - 1998 pages 97-103, XP000892641 the whole document	1-21
X	BOEHM G. ; MULLER H.: "Docosahexanoic and arachidonic acid absorption in preterm infants fed LCP-free or LCP-supplemented formula" ANNALS OF NUTRITION AND METABOLISM, vol. 41, no. 4, - 1997 pages 235-241, XP000892643 the whole document	1-21
X	ATTERBY H.: "An investigation into the effects of dietary fatty acids on intestinal activity." FOOD SCIENCE & TECHNOLOGY TODAY, vol. 10, no. 3, - 1996 pages 159-163, XP000892706 page 159, column 2 -page 162, column 2	1-14
X	MOYA M. ; JUSTE M. : "Intestinal absorption of LCPUFAs and plasma pattern in the preterm infant" PEDIATRIC RESEARCH, vol. 42, 1997, page 409 XP000892678 abstract	1-14
A	EP 0 231 904 A (MILUPA AG) 12 August 1987 (1987-08-12) abstract; claims; examples	1-21
A	EP 0 682 879 A (HEIRLER HORST) 22 November 1995 (1995-11-22) abstract; claims; examples page 2, line 2 -page 4, line 43	1,8,15
A	US 4 670 285 A (CLANDININ MICHAEL T ET AL) 2 June 1987 (1987-06-02) cited in the application the whole document	1-21

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/29478

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-7 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/29478

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		DE 69305723 T	03-04-1997
		DK 599576 T	25-11-1996
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